Connective Tissue Response to Fluorosed Bone.* (30956)

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The present research was undertaken to investigate two questions: the toxiciy of fluorine at the cellular level, and the possible action of fluoride in treatment of osteoporosis.

Irving and Handelman(1) have shown that autogenous implants of bone in rats became surrounded by giant cells which resorbed the bone, and had all the properties and enzymes of osteoclasts. In the present experiments this procedure was repeated but before the bone to be implanted was removed from the animals, some of the rats were put on to a high fluoride diet, so that when the bone was removed it had a high fluoride content. The giant cell response after implantation was compared with that in animals on a normal diet, and also the tissue response in general was examined, since it has been claimed that fluoride can reduce the growth rate of cells in tissue culture. Under these experimental conditions, no effect was found on either the formation of giant cells or the tissue response, compared with the changes in the control animals.

Material and methods. Ninety-nine young male rats of the Holtzman strain were used; 47 rats were maintained on the stock chow throughout, and 52 were kept for 3 weeks on stock chow to which NaF had been added at a concentration of 450 ppm F. At the end of this period, a portion of the scapula was removed under aseptic conditions from each rat. At this point, the rats previously on the high F diet were returned to normal rat chow. Ten scapulae from the control rats and 11 from the high F rats were analyzed for F. The remaining scapulae were devitalized by repeated freezing and thawing and were implanted under the skin of the back of the respective donor rats 2 weeks after they had been removed. The scapulae with the surrounding tissue were removed from half the control and experimental groups after 2 weeks and from the other half after 4 weeks.

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TABLE I.

F content of bone ash—ppm	Initial value	2 week implants	4 week implants
	Control animals		
Average	850	1220	1540
Highest	1240	1400	1620
Lowest	540	920	1460
	Experimental animals		
Average	9860	9390	9710
Highest	11090	10400	10500
Lowest	6100	8280	9290

Five scapulae from each group were analyzed for F and the rest prepared for histological examination.

For histology, the implants were fixed in formol-saline, decalcified in a mixture of formic acid and sodium citrate, embedded in paraffin and stained with hematoxylin and eosin. Fluoride analyses were carried out on the various bone samples by the method of Singer and Armstrong(2). The animals were weighed twice weekly.

Results. The control animals weighed an average of 51 g at the beginning of the experiment and 175 g at the end. They were in perfect health throughout. The experimental animals were also in good health but did not gain weight at the same rate as the control animals while on the fluoride regime. They weighed an average of 49 g at the beginning of the experiment and 144 g at the end.

The fluoride content of the implants is shown in Table I. There was a wide scatter of values in both the control and experimental animals; however, the difference between the two groups was so marked that it was felt that only averages and the highest and lowest figures need be given. In general the F content of the bone ash of the experimental animals was 7 to 10 times that of the controls.

As previously described(1,3), autogenous implants of bone are first surrounded by a small round cell reaction which is soon succeeded by the formation of a fibrous capsule. On the sixth day giant multinucleated cells appear and increase in number up to 2 weeks

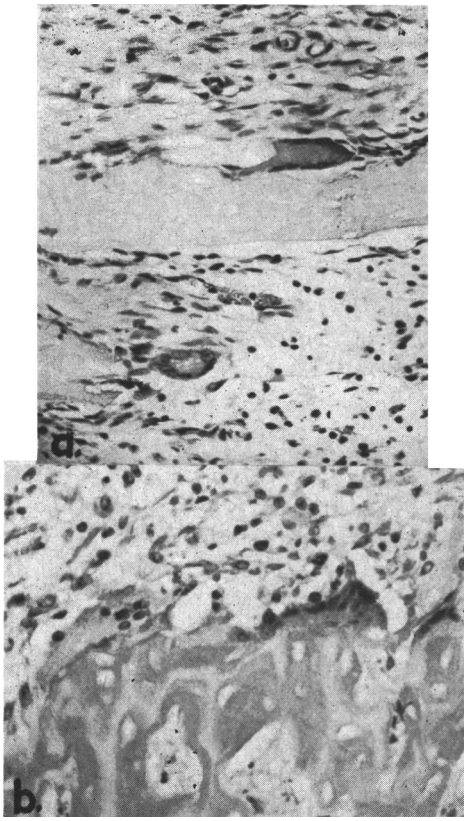

FIG. 1a. Implanted bone from a control animal removed after 2 weeks. Two giant cells can be seen. Hematoxylin and eosin. × 280.

FIG. 1b. Implanted bone from an animal previously on the high F diet, removed after 2 weeks. Two giant cells and part of a third can be seen. Note tissue response similar to that in Fig. 1a. Hematoxylin and eosin × 280.

after implantation, when they reach a maximum. In previous publications reasons have been adduced for considering these cells to be acting like osteoclasts. In the present results the control bone implants had similar giant cells around them (Fig. 1a). Precisely similar cells were also seen around the fluorosed bone (Fig. 1b). It is not possible with this technic to make a quantitative comparison of their number, but the following can be concluded: fluoridation of the bone did not prevent the appearance of these cells and judging subjectively, as many were present as were found around bone with a lower F content. No difference was seen in the tissue response. The same fibrous capsule was present, no abnormal cells were seen, nor was there any evidence of necrosis or degenerative changes. In both groups the same response was seen after 2 and 4 weeks.

Discussion. Zipkin *et al*(4) reported the F content of various bones of rats on a diet containing 0.8 ppm F. There was a certain degree of variation in F content from bone to bone. No one to our knowledge has reported the F content of the scapula. The figures given here were slightly higher than those of Zipkin *et al* for the mandible, tibia-fibula and vertebra, and possibly the rat chow used in the present experiments had a higher F content than the diet of Zipkin *et al*. The experimental animals' bone had a F content 7 to 10 times that of the control animals, which is in accord with many similar reports in the literature. The F content of the implants of the control animals seemed to rise with time while those of the experimental animals did not. Possibly these control implants, having an initially low F content, continued to gain F from the body fluids, whereas those from the experimental animals were already nearly saturated with F and thus were not able to acquire additional amounts of F.

Within recent years, fluoride has been used in the treatment of osteoporosis(5,6,7); both subjective improvement and also an increase in Ca retention have been obtained. No explanation for this effect has yet been forthcoming, but it has been claimed that an increase in bone apposition occurs. If the present results can be applied to this problem, it would appear that an increase in F content of bone from 7 to 10 times does not affect bone resorption, does not depress giant cell formation, and highly fluorosed bone is nontoxic in the surrounding tissue. Berry and Trillwood(8) found that NaF added to culture media to give an augmented F content of 0.045 to 4.5 ppm, reduced the growth rate of HeLa cells and of mouse fibroblasts. These results have been interpreted in some quarters as indicating that F at levels used in water fluoridation is toxic. Proffit and Ackerman(9) tested the effects of F in concentrations as high as 10-20 ppm on DNA and protein synthesis in organ culture of growing bone, and found no demonstrable effects. Armstrong *et al*(10) repeated some of the work of Berry

and Trillwood and were unable to detect any inhibitory effects of F on cultures of HeLa and human esophageal cells, in concentrations of up to 10 ppm. Berry and Trillwood(11) have criticized the findings of Armstrong *et al,* but the latter workers have repeated and confirmed their earlier findings(12) under conditions which have met Berry and Trillwood's criticisms.

Summary. A group of rats were placed on a high fluoride diet for 28 days. Portions of their scapulae were then removed and devitalized, and in the meantime the animals were put on a diet of normal F content. A control group of animals were similarly treated, but did not get a high F diet. The scapulae were implanted subcutaneously into the respective donor animals and removed after 2 or 4 weeks, and examined histologically and chemically. Despite an F content 7 to 10 times higher in the experimental compared to the control animals' scapulae, the tissue responses were identical: a fibrous capsule formed around the implants and giant cells appeared and began to resorb the bone. No necrosis or degeneration was seen. It is concluded that, under these conditions, F does not exert any toxic action.

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Transmural Potentials Across the Small and Large Intestine of the Bullfrog, *Rana catesbeiana.** (30957)

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In contrast to the large number of studies on mammalian intestine(1,2,3) no systematic study of the electrical characteristics of the small intestine of the bullfrog has been published. This report describes results obtained *in vitro* in a study of the electrical potential difference (PD) across both the small and large intestine of the bullfrog *(Rana catesbeiana)* and its modification by the presence of sugars and amino acids in the bathing fluids. A preliminary report of the results has been made(4).

Materials and methods. Bullfrogs were obtained from September to July from commercial suppliers. They were stored at 8-12°C for approximately 2 weeks in glass containers containing distilled water that was frequently replaced. Such storage minimizes seasonal changes. After pithing the frog, about 15 cm of the proximal small intestine was removed distal to the entry of the bile duct, everted

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